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(54) **THE EXTRACT OF PINE NEEDLE AND THE USE THEREOF**

(57) This invention discloses a kind of pine needle extract. The extract can be used to treat the following diseases: hypertension, coronary heart disease, angina pectoris, arrhythmia, diabetes, hyperlipemia, high blood viscosity, high blood aggregation, scleratheroma, cerebral infarction, brain scleratheroma, senile dementia, sudden deafness, etc.

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Description

Technical Fields:

5   [0001]   This invention relates to an extract of pine needle and its uses in medicines, especially its use in treating hypertension and coronary heart disease. This invention also relates to a pharmaceutical composition comprising the extract of pine needle and its pharmaceutical uses.

Background:

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[0002]   Pine needle is also named pine leaf. Its Latin name is Folium Pini. One important thing is that Pine needle is leaf of plants of Pinaceae and Pinus. It comes from Pinus tabuleaformis carr, Pinus massoniana Lams, Pinus Yuananensis Franch, etc. In China there are 20 species and 10 varieties of Pinaceae plants, 16 species and 2 varieties have been planted.

15   [0003]   In China, there has been a view that pine needle may be used as a supplement material for some medicines for many years. For example, some of the recipes collected in the Great Dictionary of Chinese Herbs can treat injury, edema, eczema, chronic bronchitis and asomnia, or prevent influzeza, epidemic encephalitis. Pine needle is used as one of the ingredients in these recipes .

[0004]   Although pine needle has been used as a supplement material in medicines for a long time, it has never
20   been reported that pine needle along has the effect of treating hypertension, coronary heart disease, angina pectoris, arrhythmia, cerebral infarction, hyperlipemia, high blood viscosity, high blood aggregation, arterial sclerosis, senile dementia and sudden deafness. It has never been reported that pine needle or its extract in combination of other substances can be used to treat the above diseases.

[0005] ·   After study of many years, the inventor has found that an extract of pine needle can be used to treat hyper-
25   tension, angina pectoris, myocarditis, arrhythmia, cerebral infarction, hyperlipemia, diabetes, high blood viscosity, high blood aggregation, arterial sclerosis, senile dementia, and sudden deafness. The effect is satisfactory. The new reliable uses of pine needle have been found.

Brief Description of the Invention:

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[0006]   Therefore, one objective of the invention is to provide an extract of pine needle for treating many diseases, such as, coronary heart disease (CHD), angina pectoris, myocarditis, arrhythmia, hypertension, hyperlipemia, high blood viscosity, high blood aggregation, diabetes, cerebral infarction, cerebral arterial sclerosis, arterial sclerosis, senile dementia, and sudden deafness, etc.

35   [0007]   Another objective of the invention is to provide a pharmaceutical composition for treating diseases.

[0008]   Another objective of the invention is to provide a method for treating various diseases.

[0009]   Another objective of the invention relates to use of an extract of pine needle in treating diseases.

[0010]   The other objectives of the invention will be showed in the description of the invention.

[0011]   According to the invention, an extract of pine needle has the colour of light brown to dark brown in solid state
40   and shows the maximum absorption peak near 242nm in the ultraviolet-visible light spectrum.

[0012]   An extract of pine needle is used for treating myocarditis, CHD, angina pectoris, arrhythmia, hypertension, hyperlipemia, high blood viscosity, high blood aggregation, cerebral infarction, senile dementia, dliabetes, cerebral arteriosclerosis, arterial sclerosis, and sudden deafness, etc.

45   ### Brief Description of the Drawings:

[0013]

Figure 1 shows the thin-layer chromatographic results of the extract of pine needle of this invention.

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Figure 2 shows the ultraviolet-visible light absorption graph of the extract of pine needle of this invention.

Figure 3a shows the dependent relationship between the vasodilation capability and the dose/concentration of the extract when endothelium exists.

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Figure 3b shows the relationship between vasodilation capability and dose/concentration of Capoten as the control group when endothelium exists.

Figure 3c shows the relationship between vasodilation capability and dose/concentration of the extract of pine needle when endothelium is removed.

Figure 3d shows the relationship between vasodilation capability and dose/concentration of Capoten as the control group when endothelium is removed.

Figure 4 shows synergetic effect of the extract of pine needle and the extract of Radix Puerariae when endothelium exists.

Detailed Description of the Invention:

[0014]   According to the invention, an extract of pine needle is light to dark brown in solid-state and water-soluble, and has a maximum absorption peak at about 242±1nm in ultraviolet-visible light spectrum.

[0015]   The inventor has found that the extract of pine needle has therapeutic effects in treating various diseases, including, such as, myocarditis, CHD, angina pectoris, arrhythmia, hyperlipemia, high blood viscosity, high blood aggregation, cerebral infarction, diabetes, cerebral arteriosclerosis, senile dementia, arterial sclerosis, and sudden deafness, etc. . The extract of pine needle has particularly therapeutic effects on hypertension, hyperlipemia, high blood viscosity, and high blood aggregation.

[0016]   In this invention, the extract can be extracted from pine needle using common methods. These methods include, for example, water-extraction method, water-alcohol method, alcohol extraction method and etc. Preferred is water-extraction method. The pine needle may be pine needle of various pine trees such as, Dahurian Larch. Pinus tabulaeformis carr, Pinus massoniana Lamb, Cedrus deodara (Roxb.) Loud, Pinus pumila(pull) Regel, Pinus koraiensis Sieb. et Zucc., Pinus Yunnanensis Franch, Japanese black pine and etc. The preferred pine needle is selected from Pinus tabuleaformis carr, Pinus massoniana Lams and Pinus Yunnanensis Franch.

[0017]   The extract of pine needle is light brown to dark brown in solid-state. Whether the colour is light or dark depends on the method applied for drying. The extract is soluble in polar solvents such as water and alcohol, and its maximum absorption peak is found near 242nm in the ultraviolet-visible light spectrum. Its R_f values are 0.93 and 0.78 respectively when it is identified by using a thin-layer chromatographic analysis.

[0018]   A pharmaceutical composition comprises a therapeutically effective amount of the extract of pine needle and a pharmacologically acceptable carrier or excipient.

[0019]   The pharmacologically acceptable carrier or excipient is commonly used carriers/excipients.

[0020]   A method for treating diseases, comprising the administration of therapeutically effective amount of the extract of pine needle of this invention to patients. These diseases include: hypertension, diabetes, CHD, angina pectoris, arrhythmia, myocarditis, hyperlipemia, high blood viscosity, high blood aggregation, cerebral infarction, senile dementia, arterial sclerosis, and sudden deafness.

[0021]   The extract of pine needle of this invention is compatible with other drags in treating the above or other diseases. For example, it can not only lower blood pressure, blood-lipid, blood sugar, blood viscosity and the aggregation rate of platelet, but also regulate calcium metabolism when used together with calcium supplements.

[0022]   The extract of pine needle can also be combined with other ingredients such as Radix Puerariae, Ramulus Uncariae Cum Uncis, pearl strutum powder, powder of pearl, Os Draconis, Concha Ostreae, leaf of Ginkgo biloba L., Radix Salviae Milliorrhizae, Flos Carthami, Rhizoma Chuanxiong, Fructus Trichosanchis, leaf of cypress and etc. in treating the above diseases.

[0023]   According to this invention, a formulation comprises pine needle and Radix Puerariae for treating the above diseases. The proportion of pine needle and Radix Puerariae is 3:1-5:2, preferably, 3:1-4:1. According to the invention, the formulation further includes powder. The ratio between pine needle and pearl strutum powder is 20:1-2:1. The proportion of pine needle, Radix Puerariae and pearl strutum powder is preferably 18:6:9. The regulation effect of calcium metabolism can be increased when pearl strutum powder is added to the formulation.

[0024]   Radix Puerariae is the root of Pueraria Lobata (Wild) Ohwi, and pearl strutum powder is made from the inner parts of shells of Cristavia plicata (Leach) or Aaadonta woodiana (lea.). Radix Puerariae and pearl strutum powder have wide sources are available from the market.

[0025]   According to this invention, a pharmaceutical composition comprises an effective amount of the extract of pine needle and Radix Puerariae, and a pharmacologically acceptable carrier/excipient.

[0026]   A therapeutic method comprises the administration of therapeutically effective amount of the extract of the formulation for treatment of the above diseases.

[0027]   The extract of pine needle and Radix Puerariae can be extracted from the mixture of these two herbs, and it may be obtained by mixing the extract of pine needle and the extract of Radix Puerariae.

[0028]   The pharmaceutical compositions of this invention can be delievered by oral administration or topical application. They can be produced in various kinds of dosage forms, such as, tablets, oral solutions, powders, pellets, cap-

sules, injection (including intravenous injection), lotions for external application, Gao, tincture, pulveres, and infusions, etc.

[0029]   The dosages of the extract of pine needle are, in oral administration: 1-3g per time for adults, preferably 1-2g per time, three times a day, and 0.5-1g per time, three times a day for children aged at 5-14 (above 5 years old and below 14 years old).

[0030]   Now as an example, we will describe in details the process of making the extract of pine needle by using water-extraction method.

[0031]   Take pine needle as a raw material. The pine needle can be processed in whole or be cut into segments or triturated into powder before processing. It is preferred to cut pine needle into segments before treatment. Add excess water into pine needle. The water added is at least 4 times (by weight), preferably 8-9 times, of the pine needle. The mixture is heated to boiling for extracting. The time period for boiling is 1-2 hours and preferably 1.5h. The mixture is filtered after extracting and then condensed to give a concentrated solution in dark brown.

[0032]   The residue from the filtration may be extracted for second time by being added with water and boiling. The added water is at least 3 times by weight of the residue, while 6-7 times is preferred. The time for boiling is 0.5-1.5 hours, preferably 1h. After the filtration of second time, The filtrate of the second time can be combined with the filtrate of the first time and then they can be condensed together to get the condensed solution.

[0033]   If necessary, the condensed solution can be dried to give powder in light to dark brown. Many methods such as spray drying method, vacuum drying method, and freeze drying method, etc. can be used for drying. The higher the temperature applied during the drying process, the darker the colour of the powder.

Identification test for the extract of pine needle

1. Identification test by using thin-layer chromatography (TLC)

[0034]   5g of the dry extract powder of pine needle was weighed and ethyl ether was added to immerse it for 2 times, 30ml, 1 hour for each time (keep shaking). After filtering, the filtrates of these two times were pooled together. ethyl ether was retrieved to get dry substance. A little of ethyl ether was added to dissolve the dry substance, and the solution was transferred into a volumetric flask with a capacity of 1ml. Added ethyl ether to get a solution of 1ml and shake evenly. Took the solution as the sample to be tested. Tested the solution with TLC according to the method published in the Chinese Pharmacopoea (edition: 1995, appendix VIB): Took 10-20ul of the above solution to be tested. Dot it onto the thin-layer plate of Silica G with sodium carboxymethylcellulose as the adhesive. Used petroleum ether and ethyl acetate (ratio of 4:1) as the developer. Developed the spot into 12cm. Took it out and air dry. Sprayed a solution of sulfuric acid in alcohol with the concentration of 20% on it and then heated it at 105°C until the color of the spot was developed. There were same two main spots of violet red in color among the chromatography of the tested solution. The values of R_f were 0.93 and 0.78, respectively (see the attached figure 1).

2. Identification test by using ultraviolet spectrum

[0035]   Made the extract powder of pine needle through a sieve of 60 mess. Then dried it at 50°C-55°C for 3 hours. Then moved it into the solica dryer for storage and use.

[0036]   Weighed 2g of the pre-treated extract of pine needle (Notice: The accuracy is required to be 0.01g). Put it into a triangular flask of 50ml with a plug. Added petroleum ether 14ml each time, 3 times in all and 30 minutes for each time to extract with ultrasonication. Then took the mixture for filtration. The solvent was volatilized to get the residual dregs. Added chloroform to the dregs for extraction with ultrasonication, 15ml and 30 minutes for each time, 3 times in all. Then took it for filtration. Pooled the filtrates. Retrieved chloroform to get the dry substance. Added a little of chloroform to make the dry substance dissolved. Moved the solution to a volumetric flask with a capacity of 10ml. Added chloroform to the scale. Shook it evenly and used as sample to be tested.

[0037]   Used ultraviolet spectrophotometer (Model VV-260, Shimadzu, Japan):

The width of the gap: 1nm;
The increment of wavelength: 2nm;
The range of wavelength:. 200-500nm;
The range of absorptance: 0-1.

[0038]   Diluted the tested solution appropriately to make its absorptance being 0.6-0.8 at its maximal absorption wavelength. According to the spectrophotometric method published in the Chinese Pharmacopoeia (Edition: 1995, appendix VA), the ultraviolet-visible spectrum detected in the wave length ranges from 200nm to 500nm (see attached figure 2) showed the maximal absorption peak appears at the wavelength 242±1nm.

1. The hypotensive effect of the extract from pine needle

[0039] The extracts of Pine needle (decoction, spray drying powder after concentration or water extraction-alcohol deposition) showed significant effect of decreasing blood pressure on the patients with primary hypertension or renal hypertension and the rats with spontaneous hypertension (SHRsp) or renal hypertension by one-clamp of the two renal arteries. This effect was dose-dependent.

1.1. Acute decreasing blood pressure test of the extract from pine needle:

1.1.1. Materials and methods:

[0040]

Experimental animals: 30 male Wistar rats in good health, wherein 20 of them were operated to form renal hypertension by one-clamp of one renal arteries according to Goldblatt method; 30 male rats with spontaneous hypertension (SP).
Drug: the extract power of pine needle, compound Folium Apocyni Veneti tablet.
Normal control group: administrating extract powder of pine needle, 1. 6g/kg/time.
Blank control group: (SP) administrating distilled water, 2ml/100g (body weight) /time.
Positive control group: administrating compound Folium Apocyni Veneti tablet, 1.08g/kg (body weight)/time.
Low dose group: administrating the extract powder of pine needle, 0.83g/kg (body weight)/time.
High dose group: administrating the extract powder of pine needle 3.3g/kg (body weight)/time.

[0041] Isolated the right common carotid artery after the animal was anesthetized. Ligated the distal end of the right common carotid artery and inserted the heparinized catheter into the proximal end. Connected the catheter with a five-lead physiological instrument, and monitor the blood pressure and the heart rate. Opened the abdominal cavity. Administrated the drug via the duodenum. Observed the changes of blood pressure and heart rate before administrating the drug, 30 minutes, 1 hours, 2 hours, 3 hours after administrating the drug.

1.1.2. Results: (see table 1)

[0042] (1) Blood pressure of the normal group was decreased slightly at 30 minutes after the administration, but restore to the original level at 1 hour after the administration ($P>0.05$). (2)No obvious change of blood pressure in blank control group ($P>0.05$). (3)Blood pressure of positive control group began to decrease at 30 minutes after the administration, the hypotensive peak appeared at 2 hours after the administration), the decreasing rate was up to $8.1\pm1.22\%$, and there was statistical significance compared with the blood pressure before the administration ($P<0.05$). (4) Blood pressure of the low dose group began to decrease at 30 minutes after the administration. The hypotensive peak appeared at 2 hours after the administration. The decreasing rates were up to $14.3\pm2.32\%$ (the group with renal hypertension.) and $15.6\pm2.17\%$ (the group of SP), respectively, there is very significant difference compared with that before the administration ($P<0.01$). (5) Blood pressure of the high-dose group was decreased most significantly at 2 hours after the administration. The decreasing rates were up to $18.6\pm2.07\%$ (the group with renal hypertension) and $17.6\pm1.98\%$ (the group with SP), respectively. Moreover, there are very obvious differences compared with the blood pressure of this group before the treatment ($P<0.001$) and that of the other group at 2 hour after the treatment ($P<0.001$).

Summary:

[0043] Acute decreasing blood pressure test of the extract of pine needle showed that the extract of pine needle had no obvious effect on the blood pressure of normal rats, but it could decrease significantly the artery pressure of the rats with SP and renal hypertension. The effect appeared at 30 minutes after the administration. The hypotensive peak appeared at 2 hours after the administration. The effect could last for 3 hours or more and was dose-dependent. The effect of decreasing blood pressure of the extract was stronger than that of compound Folium Apocyni Veneti tablet.

1.2. Long-term decreasing blood pressure test of the extract from pine needle.

1.2.1. Materials and methods:

[0044] 10 male Wistar rats in good health, 50 male rats with spontaneous hypertension (SP). Drug: same as that

of the acute decreasing blood pressure test.

[0045]   Methods: Wistar rats (1) normal control group: administrating the extract powder of pine needle, 1.6g/kg (body weight)/d. Every 10 SP rats made a group; (2) blank control group: administrating distilled water 2ml/100g (body weight)/d. (3) compound Folium Apocyni Veneti group: 1.08g/kg (body weight)/d. (4) Low dose group: 0.83g powder of pine needle /kg (body weight)/d. (5) moderate dose group: 1.6g powder of pine needle /kg (body weight)/d. (6) high dose group: 3.3g powder of pine needle /kg (body weight)/d. Gavage once a day. The volume of gavage was 2ml/kg (body weight) each time. 3 weeks made up a course of treatment. Examined the blood pressure and the general state of the rats before and after the drugs given. Determined the blood pressure in vitro and the carotid blood pressure, then tested the data with Linear Relation-Regression analysis. Showed the results in the form of Mean±Standard Deviation (K±SD). Then tested the difference existing between every two groups.

1.2.2. Results: (see table 2)

[0046]   After the treatment with the extract powder of pine needle, the general states of the rats were normal. Body weights of the rats in blank control group increased 2g on average while that of the rats in high, moderate and low dose groups increased 6.7g on average. The extract of pine needle had no effect on the blood pressure of normal control group. The decreasing rates of blood pressure in low dose group, moderate dose group and high dose group were 30.7±0.93% , 38.82±3.68% and 48.22±1.16%, respectively. In these three groups, there is very significant difference compared with the blood pressure before treatment. (P<0.001), while remarkable difference compared with blank control group and Folium Apocyni Veneti group (P<0.001). The MAP after the treatment showed significantly positive relation with the blood pressure in vitro (r=0.903, t=8.899 P<0.001).

[0047]   Conclusion: The extract of pine needle had a satisfactory effect of decreasing blood pressure on rats (SP). The effect was dose-dependent. The extract had no impact on normal blood pressure. Its effect of decreasing blood pressure was stronger than that of compound Folium Apocyni Veneti tablet (P<0.001). The activity and appetite the SP rats after the treatment by the extract were normal, and their body weight increased significantly.

1.3. Clinical test of the decreasing blood pressure effect of the extract from pine needle

1.3.1. Methods:

[0048]   Selected 106 in-patients and 38 out-patients for the test. After inquired the medical history intensively, gave physical examination and laboratory examination, 124 patients were diagnosed as essential hypertension (EH) according to the diagnostic standard of hypertension worked out by WHO expert association, including 38 patients of primary EH, 71 of secondary EH, 15 of tertiary EH and 20 of renal hypertension. The patients had stopped taking hypotensor, diuretic and vasodilator for more than a week before involved in the test. They were divided into two groups, that is, the treatment group and the control group.
Patients of the treatment group took orally the extract of pine needle (in the form of oral solution) 15ml each time, three times a day, one month/course of treatment, patients of the control group took compound Folium Apocyni Veneti tablet, 2 tablets each time, three times a day, one month/course of treatment. Determined the blood pressure of all the patients in the morning of three days before and after the administration, respectively. These three days must be the days other than the day on which the drug was administrated. Took the means as the blood pressure before and after treatment respectively. (Notice: every 15ml of the oral solution contained 1.6g of the extract from pine needle.)
The standard for judging the effect of decreasing blood pressure:

(1) Remarkable effectiveness: meeting either of the two requirements as follows: (1) Made the diastolic pressure normal with decreasing it by more than 10mmHg. (2) Made the diastolic pressure lowered by 20mmHg or more although the diastolic pressure didn't become normal.

(1) Effectiveness: meeting one of the three requirements as follows: (1) Made the diastolic pressure normal with decreasing it by less than 10 mmHg. (2) The diastolic pressure was 10-19mmHg lower than before treatment, but it still didn't become normal. (3) The systolic pressure was at least 30mmHg lower than before treatment.

(3) Ineffectiveness: Not meeting any of the above standards.

1.3.2. Results: (see table 3)

[0049]   There were no significant differences in the ages and the blood pressures before the treatment of these two groups (P>0.05). When compared the blood pressure before and after the treatment, the blood pressure in the treat-

ment group was decreased most significantly (P<0.001), while the blood: pressure in the control group was lowered slightly (P<0.05). There is obvious difference between the treatment group and the control group. Both of the remarkable effective rate and effective rate of the treatment group are significant that these of the control group (P<0.001 or P<0.05).

[0050] During the test, it was also observed that the symptoms such as dizziness, headache, chest distress, palpitation, insomnia, dysphoria in chest palms-soles were relieved significantly or disappeared in the treatment group. The remarkable effective rate of the treatment group was up to 52.41-75.22%, which was obviously higher than that of the control group. (P<0.001). No adverse reaction was observed. No abnormality of blood routine, urine routine, stool routine, liver function and renal function was observed. The renal function was improved more or less among the 20 patients with renal hypertension. The urea nitrogen and creatinine in blood declined and isotope renogram showed that the renal excretory function was improved than before treatment.

[0051] Conclusion: The extract of pine needle had remarkable therapeutic effect on essential hypertension and renal hypertension. Its effect of decreasing blood pressure, relieving clinical symptoms and improving renal function was remarkable super than that of the control group (compound Folium Apocyni Veneti).

2. The hypolipidemic test of the extract from pine needle.

2.1. Materials and Methods:

[0052] Experimental animals: 60 Beijing white rabbits with big ears, all male with body weight of 1.5-2kg.

[0053] Drugs and reagents: cholesterol (produced at Beijing Chemical Reagent Company). Propyl thiooxyuracil tablet (refers to PTV hereafter, produced in Germany, 50mg each tablet), lard (prepared by ourselves). The extract powder from pine needle of the invention (refers to the powder of pine needle hereafter). Inostitol hexanicotinate tablet (produced by the second Pharmaceutical Factory of Beijing, 0.2 each tablet). The kits of total cholesterol, triglyceride, high density lipoprotein, and low density lipoprotein (produced by Beijing Zhongsheng Biotechnical Company).

[0054] Methods: The white rabbits with big ears were divided into 5 groups at random after raised for 10 days with normal diet. 10 rabbits each group. The high fat diet was given after taking blood from the helicine veins of the fasting rabbits in the morning. Determined the blood-lipid of the blood samples. The high fat diet included cholesterol 2g/kg (body weight)/d, lard 0.5g/kg/d, PTV 12.5mg/kg/d. In the forth group, gave 075g/kg/d of the powder of pine needle along with the high fat diet to the rabbits of (the prevention group). Took blood from the fasting rabbits again and determined their body weights after 15 days of high-fat diet. Then stopped the high-fat diet and changed it for normal diet: (1) blank control group: giving distilled water 10ml each day with the gavage method; (2) positive control group: giving Inostitol hexnicotinate tablets, 0.3g/kg/d; (3) low dose group: giving the powder of pine needle, 0.25g/kg/d; (4) prevention group: went on giving the powder of pine needle 0.75 g/kg/d, 30 days in all; (5) high dose group: giving the powder of pine needle, 1.5g/kg/d. The powder was dissolved in 10ml distilled water and gave it with gavage method once a day. Took blood from the fasting rabbits on the fifteenth day after the administration to determine the levels of blood-lipid. The levels of total cholestorol, triglyceride, high density lipoprotein and low density lipoprotein were examined by CHOO-PAP method, GPO-PAP method, PTA-Mg^{2+} deposition method and PVS one-step deposition method, respectively. The instrument used was Hitachi, Model 750 automatic biochemical analyzer.

2.2. Results: (see table 4)

[0055] From table 4, we could learn that there was no statistical difference among various indexes of the blood-lipid of all the groups before the high fat diet given (P>0.05). However, after the high fat diet given, the levels of total cholestovol, triglyceride, and low deasity lipopratein in Group 4 (prevention group) were obviously lower than that in the other four groups, and the differences showed remarkable statistical significance (P<0.001). The levels of total cholesterol, triglyceride and low density lipoprotein were decreased after the treatment in all the groups. Remarkable statistical significance existed when compared their levels of Group 1 with that of the other four groups after the treatment. Their levels after treatment were lowered most obviously in Group 4 (P<0.001). The decreasing rate of Group 1 was significantly different from that of the other four groups. The decreasing rates in groups 3, 4 and 5 is super compared with that in Group 2. The decreasing rate of triglyceride in Group 2 showed no statistical difference when compared with that in Group 1, but showed statistical difference when compared with that in Group 3, 4, 5, and wherein the most significant difference appears in Group 5 (P<0.001). The decreasing rates of low density lipoprotein in Group 3, 5 were most significant (P <0.001). The levels of high density Lipoprotein declined in Groups 1 and 2, while increased in Groups 3, 4 and 5 after the treatment, wherein the increment in Groups 4 and 5 are more significant, while that in Group 5 was most significant (P < 0.001).

[0056] Conclusion: For the animals with hyperlipemia due to high fat diet, the extract of pine needle could lower the levels of serum total cholesterol, serum triglyceride, serum low density lipoprotein, while made the levels of serum high

density lipoprotein increased. The therapeutic effect was most satisfactory in the prevention group, then followed by the high dose group. All of these proved that the extract of pine needle had therapeutic and preventive effect on hyperlipemia.

5   3. The test for the extract of pine needle to regulate parathyroid function.

3.1. Materials and Methods:

[0057]   Experimental animals: 5 male wistar rats in good health with body weight of 320-360g; 10 male SP rats with
10 body weight of 170-270kg. Experimental reagents: the extract powder of pine needle, the radio-immunity kits of parathonmone (PTH) and calcitonin (CT) produced at Rongyan Company of Japan.
[0058]   Methods: The animals were divided into: ① normal control group; ② SP blank control group; ③ SP treatment group, five rats a group. Wistar rats is (1) normal control group: gave the powder of pine needle, 1.5g/kg (body weight)/d. SP rats were divided into 5 groups, 5 rats a group; (2) blank control group: gave distilled water 2ml/100g
15 (body weight)/d with the gavage method; (3) compound Folium Apocyni Veneti group: 1.08g/kg (body weight)/d; (4) Low dose group: gave the powder of pine needle 0.8g/kg/d. (5) moderate dose group: gave the powder of pine needle 1.5g/kg/d; (6) high dose group: gave the powder of pine needle 3.0g/kg/d, once gavage a day, the volume of gavage fluid was 2ml/100g (body weight). 3 weeks/a course of treatment. Made the rats anesthetized and took blood from the eyeball vein before and after the treatment. Isolated the serum by freezing and centrifugation the blood (-4°C). The
20 serum PTH and serum CT were determined using radio-immunity method.

3.2. Results: (see table 5)

[0059]   From table 5, we could learn that before treatment, significant difference existed between the values of PTH
25 and CT in Group 1 (normal control group) and that in Groups 2 and 3. After the treatment, the levels of PTH declined significantly while that of CT increased remarkably in Group 3. But as to the levels of PTH and CT, difference still existed between Group 1 and Groups 2 and 3 (P<0.05). After the treatment, significant difference existed between the decreasing values of PTH and the ascending values of CT in Group 3 and these in Group 1 and 2. (P <0.05 and P<0.001).

30 Conclusion:

[0060]   The extract of pine needle has an effect in a certain extent on the parathyroid function of SP rats. The PTH levels of SP rats declined while the CT levels increased significantly after 3 week's treatment. Although after the treatment, difference existed comparing with that in normal control group, statistical significantly difference existed when
35 compared the decreasing rate of PTH and ascending rate of CT with that in blank control group. All of these proved that the extract could regulate the parathyroid function of SP rats to some degree.

4. The effect of the extract from pine needle on the hemorheology.

40 4.1. The test for the extract to inhibit platelet aggregation

4.1.1. Materials and Methods:

[0061]   Experimental animals: 25 male Beijing white Rabbits with big ears in good health, their body weights 1.5-
45 2kg.
Reagents and Instruments: Extract of pine needle, Danshen (Radix Salviae Miltiorrhizae injection solution, containing 2g crude drugs each ml), the extract of Radix Puerariae, containing 2g crude drugs/ml), ADP (20ug/200ul), Phosphate buffer (0.2mol/l, 300mg/ml), Sodium citrate solution (3.8%), 2% silicone-petroleum ether solution, Model MG-576 platelet aggregation instrument, Desk model balancing automatic recorder, Round colorimetric cup (Model: CHRONO-
50 LOG312#), silicified centrifuge tube, Microsampler.
[0062]   Methods: Took 3ml blood from the helicine veins of the rabbits with empty stomach for 10-14h. Put blood into silicified centrifuge tube. The proportion of the blood and sodium citrate solution was 9:1. Mixed them and took the mixture for centrifugation (800rpm). After 4 minutes, took out the upper layer fluid which was platelet-rich plasma (PRP). The residual blood was centrifuged (3000rpm) for 10 minutes. Took out the supernatant which was plasma-pool platelet
55 (PPP). Tested the platelet aggregation ability with BORN turbidimetry at constant temperature of 37°C. The volume of plasma in the colorimetric cup was 250ml. The test time was 5 minutes. Samples were divided into several groups: ① ADP + phosphate buffers, ADP 2umol/L, phosphate buffer 0.2ml/L; ② ADP + Radix Salviae Miltiorrhizae injection solutio with the same volume; ③ ADP + the extract solution of Radix Puerariae with the same volume; ④ ADP + the extract

solution of pine needle with the same volume; ⑤ ADP + the composition solution with the same volume.

2. Results (see table 6)

[0063]   From table 6, it could be observed that the platelet aggregation rates of Groups 2, 3, 4 and 5 declined obviously and their decreasing rate, especially groups 4 and 5, had very significantly difference compared with that of Group 1. The 1' rate of aggregation inhibition was up to 74.56-80.06%. The 5' maximal rate of aggregation in the Groups 4 and 5 also showed significant difference compared with that in the Groups 2 and 3.

3. Conclusion:

[0064]   The test of platelet aggregation inhibition in vitro showed that the composition, the extract of pine needle and the extract of Radix Puerariae could inhabit the platelet aggregation obviously, which were significantly stronger than that of the Danshen solution of the same concentration. Their effects of inhibiting platelet aggregation were the strongest at 1'. The strength of the effects of inhibiting platelet aggregation in turns: composition group > the extract of pine needle group > the extract of Radix Puerariae group> Danshen group. The test showed that either the composition consisting of pine needle and Radix Puerariae or the extract of pine needle had important impacts on preventing platelet aggregation and treating blood stasis.

4.2. Clinical test for the extract of pine needle to improve hemorheology

1. Materials and Methods:

[0065]   87 patients aged 37-75 took part in the test, including 52 male patients and 35 female patients, wherein 36 patients with coronary heart disease, 37 patients with hypertension, 9 patients with cerebral infarction, 45 patients with hyperlipomia, 12 patients with diabetes (some patients suffered from 2 diseases or more). The control group included 44 patients, wherein 18 patients with CHD, 21 patients with hypertension, 3 patients with cerebral infarction, 32 patients with hyperlipemia (Some patients suffered from 2 diseases or more). The patients had stopped taking the drugs which had effects on the hemorherlogy such as dipgridamok, Danshen, Aspirine, Ca^{2+} -blocker agent for more than a week before taking part in the test.
[0066]   The oral solution of the extract from pine needle was given 15ml (containing 1.6g the extract of pine needle) each time, three times/day, one month /a course of treatment, in the treatment group. 3 tablets each time of the compound Danshen tablet were given, three times/ day, one month/a course of treatment, in the control group. Determined the indexes of hemorheology before and after the treatment. Tested the statistical significance of the means of the results. T-test was used to determine the statistical significance of the means of every two groups.

4.2.2. Results:

[0067]   After the treatment, erythrocyte sedimentation rate (ESR) was slowed down and haematocrit was reduced. The specific viscosity of plasma and that of whole blood (high shear, low shear)were improved remarkably. The rate of platelet aggregation was lowered significantly (see table 7 and table 8).

Conclusion:

[0068]   The extract of pine needle had obvious therapeutic effect on the patients and animals with hyperviscosity or hyperaggregation. It could reduce the ESR, haematocrit, specific viscosity of plasma and whole blood as well as the platelet aggregation rate. This was very important for quicken the blood and transform stasis, and for preventing or treating thrombotic diseases as well as improving the microcirculation. Thus, this provided the theoretical basis was for the drug to treat myocardiac inforction, coronary heart disease, Angina pectoris, cerebral infarction, hypertension, thrombotic diseases of arteria and vein, sudden deafness, and blood stasis.

5. Clinical test for the extract of pine needle to enlarge the coronary artery and against angina pectoris, arrhythmia

[0069]   42 patients with coronary heart disease took part in the test, gave each of them the extract of pine needle 15ml (containing the extract of pine needle 1 .6g) each time, three times a day. Examined the EKG, blood-lipid, TXB_2, PGl_2 before and after the treatment. The patients in the control group took orally isosorbide dinitrate 10ml each time, three times a day, one-month a course of treatment.

Results: (see tables 9, 10 and 11)

[0070]   Among the 42 patients with CHD in the treatment group, 7 patients were suffering from frequent ventricular premature beats, which the premature beats of 6 patients of them were reduced obviously and 1 patient was not effected after the treatment with the extract; 7 patients with frequent atrial premature beats: the obvious effect was showed in one case; 24 patients with chronic coronary insufficiency: the St-T segments of ECG were improved in 16 cases; 4 patients with chronic coronary insufficiency accompanied by remote mycardial infaction: the obvious therapeutic effect was showed in 1 case since that the ECG became almost normal, improved ST-T segments of EGG were showed in 2 cases, no effect was showed in the residual one case; the blood sugar and the urine sugar of the 15 patients with diabetes were reduced significantly ant their sympotoms such as limb numbness, thirsty, diuresis were improved remarkably.

[0071]   To control angina pectoris: The total incidences of angina pectoris among the 42 patients in the treatment group were reduced to 128 times a week after the treatment from 395 times a week before the treatment (P<0.001). None of them showed aggravated symptoms. The total effective rate of the treatment group was up to 92.8%. The total incidences of angina pectoris in the control group were reduced to 108 times a week after the treatment from 215 times a week before the treatment. (P<0.05). The total effective rate of the control group was up the 86.6%.

[0072]   Conclusion: The extract of pine needle could reduce the incidences of angina pectoris significantly, improve the ischemic change of ECG, control the incidences of arrhythmia (ventricular premature beats), lower the level of serum total cholesterol significantly, lower the level of serum triglyceride slightly, raise the level of serum high density lipoprotein, reduce the level of TXB_2, and raise the level of PGI_2. Therefore, the extract could increase the blood flow volumes of cerebral arteries, reduce the mgocardial oxygen consumption volume and reduce the mgocardial post-load so as to protect the ischemic mgocardium.

6. The effect of the extract from pine needle on inhabiting senility and increasing cerebral blood flow

[0073]   25 patients with cerebral infarction or senile dementia were received the treatment of oral solution of pine needle. Examined the REG (Rheology Encephalogram, REG), serum lipid peroxide and serum copper-zinc superoxide dismutase (refers to SOD_1 hereafter) before and after the treatment. The method for administration was as follows: 15ml (containing the extract of pine needle 1 .6g) each time, three times a day, 3 months a course of treatment. After the treatment, the REG was improved significantly, the level of SOD_1 was decreased and that of lipid peroxide was reduced remarkably. (see table 12)

[0074]   The REG before the treatment showed the increased vascular resistance, the reduced volume of blood flow, the .slowed blood flow rate and the decreased vascular elasticity. However, among the 25 patients who received the treatment of pine needle, 20 patients showed remarkable improvement of the REG, including reduced vascular resistance, accredited blood flowing, increased volume of blood flow and improved wave shape. All of these proved that the extract of pine needle could increase the volumes of cerebral blood flow.

7. The blood vessel-dilation effect of the extract from pine needle

[0075]   The isolated perfusion test of dilating vascular circle proved that the extract of pine needle could expand arteries. The effect was endothetium-dependent and dose-dependent. When endothelium existed, the higher the dose of the extract, the stronger the effect of vascular-dilation. The maximal dilation rate was up to 86.88±12.03%. Without endothelium, the extract could only expand the vessel slightly even at the highest concentration (14.23±2.06%). These proved that the extract of pine needle expanded the blood vessel by regulating vascular active substances of endothelium (see attached figure 3a-3d).

Example 1

Preparation of the oral solution of pine needle:

[0076]   Took 10kg pine needle, added 80kg water to it. Decocted the mixture for 1 .5h. Took out the decocted soup. Added 60kg to the decocted dregs. Decocted the mixture for 1h. Took out the decocted soup of the second time. Combined the soup of there two times together. Filtered the soup. Added alcohol to the filtrate. After standing the mixture for 24 hours, discarded the sediment, retrieved the alcohol. Concentrated the liquid into a solution of 10kg. Added distilled water to dilute it into a solution of 20kg. Added a little of stevioside and the preservative nipagin to the soup. Packed the solution into the bottles. Each bottle contained 10ml the solution.

Example 2:

Preparation of the capsules of pine needle:

[0077]   Took 10kg pine needle and 3.5kg Radix Puerariae. Added water of 5 times volume. Decocted the mixture for 2 hours. filtered the mixture. Then took out the dregs of decoction and added water the volume 4 times. Decocted the mixture for 1 .5h. Filtered it to get the soup. Combined the soup of these two times together. Added alcohol to the combined soup. Discarded the sediment after standing the mixture for 24h. Retrieved the alcohol. Then concentrated the soup into an extractum. Dried the extractum to get the dry extractum. Smashed it into powder for later use. Then 5kg pearl stratum power and smashed it. Sieved it into fine powder. Mixed it with the above powder of the extractum. Used the automatic capsule fill machine to fill the capsules with the powder. Each capsule contained 0.5g drug powder. Packed it with plastic-aluminium foil.

Example 3:

Preparing method:

[0078]   Took 1800g pine needle and 600g Radix Puerariae. Added water to the mixture of them. Decocted the mixture for two times: 8 times volume of water for the first time and decocted the mixture for 1.5h; 6 times volume of water for the second time and decocted the mixture for 1.5h. Filtered the mixture. Combined the filtrate of these two times together. Dried the combined filtrate to get the powder.
Now we will describe the animal test and the clinical test of the composition consisting of the extracts from pine needle of the invention and that of Radix Puerariae (refers to "the composition" hereafter).

8. The effect of the composition to expand blood vessel

[0079]   The isolated perfusion test of the vascular circle proved that the composition had effect of expanding arteries. This effect was endothelium-dependent and dose-dependent. With the endothelium, the higher the dose of the composition, the stronger the effect of expanding the blood vessel. The maximal expanding rate was up to 96% (see figure 4).

9. The clinical test for the composition to lower blood pressure.

9.1. Materials and Methods:

[0080]   Observed subjects: 44 patients with essential hypertension. All of them were adults aged below 60, their average age was 53±12.7. All of them were diagnosed as hypertension according to the diagnostic standard of WHO. Selected another 44 patients with essential hypertension at random as the control group. The patients of the control group were treated with Captopril. The patients of the control group were adults aged below 59, and their average age was 47±7.8. The sex and the course of disease of these two groups were listed in table 13. The patients stopped taking hypotensor a week before taking part in the test. Treatment method: gave the patients of the treatment group 1.5g composition each time, 3 times a day, and 5 days in all. If the blood pressure was not reduced to the normal scope, then a dose (0.5g) was increased every 5 days. The crescendo every 5 days, and the maximal dose couldn't exceed 3g each time and 3 times a day. The whole course of treatment was 20 days. 25mg Captopril was given to the patients of the control group each time and three times a day. If the blood pressure was not reduced to the normal scope, then a dose (12.5mg) was increased every 5 days. The crescendo every 5 days, and the maximal dose couldn't exceed 75mg each time and three times a day. The whole course of treatment was 20 days too.
The blood pressure before treatment: All the patients stopped taking hypotensor a week before treatment. Then the blood pressure was measured once a day continuously for 5 days. Took the mean of the 5 values (non same day) as the blood pressure before treatment.
The blood pressure after treatment: Measured the blood pressure once a day after the treatment. Took the mean of the 5 values measured on the 16th, 17th, 18th, 19th, 20th day after the treatment as the blood pressure after the treatment. Examined the blood routine, the urine routine, the functions of liver, the renal function, the serum electrolyte (including K^+, Na^+, Cl^-), the blood-lipid, the blood sugar, and ECG of every day before and after the treatment.

The criteria of therapeutic effect:

[0081]

Obvious effectiveness: The diastolic pressure was reduced more than 1.33kpa and become normal or it was reduced more than 2.67kpa after treatment.
Effectiveness: The diastolic pressure was reduced of less than 1.33kpa but it became normal, or it was reduced of 1.33-2.53kpa. As to the systolic hypertension, the systolic pressure was reduced more than 4kpa.
Ineffectiveness: The decrement of the blood pressure after the treatment didn't achieve the above standard.

2. Results:

[0082]   Analysis of the therapeutic effect: The blood pressures and the decrements before and after the treatment were compared and listed in Table 14. Both of the groups had very significant difference (P< 0.01) by T test with own control. But no significant difference existed between the two groups (P>0.05). Moreover, there was not significant difference between the effects of decreasing the blood pressure of the two groups after the treatment (see table 14). The changes of ECG and blood biochemistry before and after the treatment: Before the treatment, there were 10 cases with ventricular premature beats, 8 cases with atrial premature beats, 4 cases with absolute left bundle branch block, 7 cases with absolute right bundle branch block, 10 cases with left ventricular hypertrophy, 16 cases with ischemic like changes on ST-T segment of lead II, III, avF, V_4-V_6. The total of the patient foe two groups was 88. After the treatment, improved ST-T segment of lead V_4-V_6 was showed in the ECG of 12 patients, the atrial premature beats of 8 patients and the ventricular premature beats of 7 patients disappeared, and no obvious difference was showed in blood electrolyte (K^+, Na^+, Cl^-) and blood sugar. 3. Conclusion: The composition could lower the essential hypertension and the renal hypertension significantly. The effect of lowing blood pressure appeared at 30 minutes after the administration and reached the peak at 2 hours after the administration. The effect would last for more than 3 hours and was dose-dependent. The blood pressure would remain normal for 20 hours after the drug withdrawn. It could lower the levels of blood-lipid concurrently. Therefore the composition was especially suitable for those with hypertension accompanied by hyperlipidemia. No adverse effect was observed among the 44 patients treated with the composition. The total effective rate was 77% (see table 15). The clinical test of the formulation which consists of the extract of pine needle of the invention, the extract of Radix Puerariae, and pearl stratum power (refers to: "formulation" hereafter) will be disclosed as follows: 10. The observation of the therapeutic effect for the formulation to treat myocarditis

Subjects and Methods:

[0083]

1. Treatment group: 11 patients with viral myocarditis and 5 patients with rheumatic myocarditis were selected as the treatment group (16 cases in all) according to the diagnostic criteria of myocarditis, including 9 male and 7 female aged 6-17, whose average age was 11.6±2.8, 10 cases with the ECG of showed myocardial ischemia, 9 cases with arrhythemia (wherein 6 cases with tachycardia, 4 cases with frequent premature beats, and 2 cases with bradycardia).

2. Control group: 12 patients were selected as the control group, including 8 cases with viral myocarditis, 4 cases with rheumatic myocarditis. There were 5 male cases and 7 female cases aged between 4 and 17, their average age was 9.1±2.6. The ECG of 9 cases showed myocardial ischemia, 7 cases showed arrhythemia (wherein 5 cases with tachycardia accompanied by frequent premature beats, and 2 cases with bradycardia).

3. Took blood from the fasting patients after the treatment: ESR, GOT, CPK, CPK-MB, ECG and RF (rheumatoid factor) were examined.

4. 1.5g of the formulation was given to the patients of the treatment group each time, three times a day. In the control group, the conventional method was used: 10% Glucose solution 500ml + Vit C 3.0g + ATP 40mg + coenzyme 100u were administrated by intravenous drip once a day, and coenzyme Q_{10} by muscular injection once a day, 30 days a course of treatment. The patients with rheumatic myocarditis in the two groups were reviewed conventional antirheumatics treatment.

[0084]   The criteria for therapeutic effect and the statistical treatment Remarkable effectiveness: all of the indexes including ESR, GOT, CPK, CPK-MB, ECG, RF and so on restore normal.

[0085] Effectiveness: the above indexes were obviously improved after the treatment, but didn't restore normal (ECG still showed abnormality of heart rate, frequent premature heats or slight changes of ST-T segments).

[0086] Ineffectiveness: the above indexes were improved to some degree, but still remained obviously abnormal.

[0087] T-test for significance was used.

[0088] Results: The comparison of the therapeutic effects between the two groups was showed as follows (see table 16):

11. The therapeutic effect analysis for the formulation to treat hyperlipidemia:

[0089] The patients with hyperlipidemia but without diabetes and liver diseases or renal diseases were selected as the observed subjects. During the observation, all of the patients stopped taking the medicines had effects on the lipid metabolism but still kept the diet as usual.

11.1. Clinical data: 110 cases were divided into the treatment group with 70 cases and the control group with 40 cases. There were 50 male cases and 20 female cases aged between 40 and 67 in the treatment group, and their average age was 55. There were 41 cases with hypercholesterolemia (TC) and 55 cases with hypertriglyceridemia (TG). In the control group, there were 28 male cases and 12 female cases (total 40 cases) aged between 40-66 and their average age was 54, including: 30 cases with hypercholesterolemia and 30 cases with hypertriglycoridemia. The two groups were comparable in sex, age and symptom.

11.2. Groups and therapeutic method

11.2.1. The treatment group: the formulation 1.5g each time, three times a day, one month a course of treatment.

11.2.2. The control group: the soft capsule of erythrinus eveningprimrose root oil, 2 capsules each time, three times a day (Notice: 0.5g each capsule, the drug was produced at the sixth pharmaceutical factory of Wuxi), one month a course of treatment.

11.2.3. Observation method: blood routine, urine routine, liver function, renal function especially the blood lipid and ECG of all the patients were examined before and after the treatment.

11.3. Results:

11.3.1. The therapeutic effect of hypercholesterolmia (TC):
Among the 41 cases in the treatment group:

Remarkable effectiveness: 20 cases,
Effectiveness: 16 cases,
Ineffectiveness: 5 cases.
The total effective rate: 87.8%;

Among the 30 cases in the control group:

Remarkable effectiveness: 4 cases,
Effectiveness: 8 cases,
Ineffectiveness: 18 cases,
The total effectiveness rate: 40%.

There is significant difference between the two groups (X^2=18.16 P<0.01). The difference of TC before and after the treatment were 2.46±0.69mmol/L in the treatment group and 0.84±0.33mmol/L in the control group, respectively. The effect of lowering TC in the treatment group was much stronger than that in the control group (t = 11.86 P<0.001). In the treatment group, if TC before treatment was compared with that after treatment, then t=22.829 P<0.001, which showed the effect of lowering TC in the treatment group was reliable.

11.3.2. The therapeutic effect of hypertriglydemia (TG):
Among the 55 cases in the treatment group:

Remarkable effectiveness: 24 cases,

Effectiveness: 25 cases,

Ineffectiveness: 6 cases.

The total effective rate: 89.1%.

Among the 30 cases in the control group:

Remarkable effectiveness: 9 cases,

Effectiveness: 5 cases,

Ineffectiveness: 16 cases.

The total effective rate: 46.7%.

There is significant difference between the two groups (X^2=18.04, P<0.01). The difference of TG before and after the treatment were 1.57±0.52mmol/L in the treatment group and 1.01±0.31mmol/L in the control group, respectively. The effect of lowering TG in the treatment group was much stronger than that in the control group (t=5.385 P<0.001). In the treatment group, if TC before treatment was compared with that after treatment, then t=22.391 P<0.001, which showed the effect of lowering TG in the treatment group was reliable.

No adverse effect was found during the treatment by the formulation. Furthermore, there was no obvious change of blood routine, urine routine, liver function, renal function and ECG before and after the treatment.

11.3.3. Conclusion:

The total effective rates for the formulation to treat hypercholesterolemia and hypertriglyceridemia were 87.8% and 89.1%, respectively, which significant difference existed comparing with that in the control group. These proved that the effect for the formulation to treat hyperlipeasa was much better than that of the control drug and had reliable effect of decreasing the TC and TG. If compared the effect of the formulation with that of the control drug by statistical treatment, then P <0.001. This proved that its effect to lower blood lipid was super to the control drug.

12. The clinical test of the formulation to treat carliovascular disease

12.1. Subjects and Materials:

12.1.1. Subjects: According to the diagnostic standard of WHO, the patients with essential hypertension, angina pectoris resulted from CHD, hyperlipemia accompanied by high blood viscosity were selected as the observed subjects, which were divided into three groups.

12.1.1.1. Group of hypertension: Total 62 cases, male: 37 cases, female: 25 cases. The ages were between 27-74. The average age was 52.3±26.5. Their medical histories lasted 2.9±3.12 years on average. Primary hypertension: 18 cases (29%), Secondary hypertension: 40 cases (64.5%), Tertiary hypertension: 4 cases (6.5%).

12.1.1.2. Group of angina pectoris: total 40 cases, male: 21 cases, female: 19 cases. The age was between 45-79. The average age was 59.8±15.2. Stable labor angina pectoris: 26 cases (65%), Unstable angina pectoris: 14 cases (35%).

12.1.1.3. Group of hyperlipemia and hyperviscosity: total 65 cases, male: 42 cases, female: 18 cases. The average age was 55.3±5.0.

12.1.2 Methods:

12.1.2.1. The therapeutic method: The patients stopped taking hypotensor, anti-angina agent and antilipemic agent, and took 1-1.5g of the formulation each time, three times a day, 4-6 weeks a course of treatment. The patients accompanied left ventricular hypertrophy were combined with Enalapril maleate, the patients attacked by angina pectoris were administrated the nitrates or Suxiaojiuxingwan, while kept the other conventional treatment unchanged.

12.2. Results

12.2.1 Group of hypertension: See table 17, 18 for the changes of systolic blood pressure (SBP) and

diastolic blood pressure (DBP) before and after the treatment.

12.2.2. Group of angina pectoris: See table 19, 20 for the therapeutic effect on symptoms, ECG and various angina

12.2.3. Group of hyperlipemia accompanied hyperviscosity: see table 21 for the changes of blood-lipid and hemorheology before and after the treatment.

12.3. Discussion:

The clinical therapeutic effect for the formulation to treat hypertension, CHD, angine pectoris, and hyperlipemia accompanied by high blood viscosity was satisfactory. The SBP and DBP were reduced by 23.4% and 20.5%, respectively. The total effective rate was 96.8%. The effective rates for the improvement of symptom and ECG of the patients with angina were 95% and 65% respectively. The effective rates of stable labor angina and unstable angina were 100% and 85.7%, respectively. The levels of CH, TG, LDL-C were reduced by 18.0%, 27.2%, 39.2%, respectively, however, the level of HDL-C was increased by 58.8%. Hematocrit, whole blood specific viscosity and plasma specific viscosity were reduced by 27.1%, 16.6% and 24.6%, respectively. There are significant or very significant difference (P< 0.05 or 0.01) in all kind of the above indexes between before and after the treatment. This proved that the formulation could lower the levels of blood pressure, cholesterol, triglyceride, LDL-C, hematocrit, whole blood specific viscosity and plasma specific viscosity while increase that of HDL-C, protect the heart and relieve angina etc.

Table 1. Acute Effect for the Extract of Pine Needle to Lower Blood Pressure of Rats with Hypertension (mean artery pressure MAD ±SD) mmHg

Groups	n	before drug given	30 minutes after drug given		1 hour after drug given		2 hour after drug given		3 hour after drug given	
		MAP	MAP	descending rate %	MAP	descending rate %	MAP	descending rate %	MAP	descending rate %
①normal control	10	111.8 ±1.73	106.2 ±1.09	5.0 ±0.48	110.7 ±1.16	1.0 ±0.08	110.9 ±1.42	0.8 ±0.02	111.5 ±1.33	0.3 ±0.01
②blank control	10	202.58 ±3.86	203.6 ±3.77	-0.5 ±0.06	204.3 ±3.81	-0.9 ±0.05	203.9 ±4.12	-0.7 ±0.04	203.1 ±3.79	-0.3 ±0.02
③group given with Folium Apocyni Veneti	10	198.7 ±4.33	192.3 ±4.67	3.2 ±0.62	186.4* ±4.08	6.2* ±0.81	182.4* ±4.33	8.1* ±1.22	188.1 ±4.29	5.8 ±0.92
④low dose group with renal hypertension	10	164.7 ±3.24	154.3* ±3.06	6.8* ±1.13	146.2** ±3.72	11.2** ±2.11	141.1** ±4.10	14.3** ±2.82	146.7** ±3.76	10.9** ±1.71
SP	10	201.6 ±3.41	186.0* ±4.03	7.6* ±1.41	174.6** ±3.77	13.4** ±1.76	170.1** ±3.23	15.6** ±2.17	178.4** ±4.06	11.5** ±1.30
⑤high dose group with renal hypertension	10	172.3 ±4.68	156.4* ±3.09	9.2* ±1.66	146.7** ±4.02	14.9** ±1.82	140.2** ±3.92	18.6** ±2.07	148.7** ±3.71	18.7** ±2.01
SP	10	206.5 ±4.02	182.2** ±3.62	11.8** ±1.73	174.1** ±4.01	15.7** ±1.96	170.2** ±3.75	17.6** ±1.98	176.3** ±4.15	14.6** ±2.10

*P < 0.05   **P < 0.01

Table 2. Long-term decreasing blood pressure effect of the extract from pine needle on SP rats

Groups	n	blood pressure before treatment ±SDmmHg	Blood pressure after treatment ±SDmmHg	decreasing rate ±SD %	Arterial blood pressure after treatment ±SDmmHg	mean arterial blood pressure after treatment±SDmmHg
①normal control group	10	102.4 ±2.38	108.0 ±2.41	-0.6 ±1.22	120.6/92.4 ±3.02/ ±1.0	101.8 ±2.04
②blank control group	10	188.8 ±5.85	189.6 ±6.68	-0.4 ±1.19	216.8/176.2 ±6.16/ ±6.47	180.5 ±6.08
③ Folium pocyni Veneti group	10	192.0 ±2.28	170.0 ±4.1	11.52* ±1.26	202.2/160.0 ±5.99/ ±4.2	174.1* ±4.73
④low dose group	10	188.7 ±3.88	130.8*** ±2.58	30.7*** ±0.93	160.4/126.6 ±3.19/ ±2.79	187.6*** ±2.79
⑤moderate dose group	10	197.6 ±5.91	120.0*** ±4.47	38.82*** ±3.68	150.0/119.8 ±3.99/ ±1.20	128.9*** ±1.93
⑥high dose group	10	194.0 ±2.28	102.4*** ±4.58	47.22 ±1.16	128.7/96.8 ±5.01/ ±3.11	107.4*** ±4.75

*P<0.05   ***P<0.001

Table 3. The therapeutic effect of the extract from pine needle on patients with hypertension

Groups	Case numbers	age	blood pressure before treatment	blood pressure after treatment	Remarkable effective rate %	effective rate %
treatment group	85	52.4 ±5.62	186.1/118.1 ±3.82/±3.82	136.3**/90.1*** ±4.06/±3.11	53.44***	86.36*
control group	56	51.1 ±6.33	178.8/114.8 ±5.64/±3.61	162.37*/106.3* ±5.42/±4.06	24.69	67.38

*P<0.05   **P<0.01   ***P<0.001

Table 4. The effect of the extract from pine needle
on the blood-lipid of the animals with hyperlipemia ($\bar{X} \pm SD$)

Group	total cholesterol				triglyceride				high density lipoprotein				low density lipoprotein			
	before HFD	after HFD	After treatment	DR %	before HFD	after HFD	after treatment	DR %	before HFD	after HFD	after treatment	IR %	before HFD	after HFD	after treatment	DR %
1)blank control group n=7	1.37 ±0.098	28.89 ±1.44	25.24 ±1.64	4.77 ±8.39	1.16 ±0.05	2.06 ±0.17	1.71 ±0.14	17.30 ±1.37	0.56 ±0.05	0.79 ±0.08	0.68 ±0.03	-11.64 ±6.53	0.91 ±0.11	8.90 ±0.49	8.22 ±0.88	8.78 ±4.06
2)positive control group n=10	1.29 ±0.13	26.67 ±1.57	19.48* ±0.88	28.20 ±4.27	1.07 ±0.07	1.87 ±0.90	1.64** ±0.11	16.03 ±2.76	0.65 ±0.09	0.83 ±0.06	0.74 ±0.05	8.90 ±3.71	1.21 ±0.25	9.20 ±0.42	5.71* ±0.37	37.38* ±5.33
3)low dose group n=10	1.42 ±0.12	27.36 ±1.60	15.46 ±1.60	43.49*** ±5.34	1.15 ±0.11	2.03 ±0.16	1.26 ±0.18	37.93 ±10.61	0.62 ±0.03	0.89 ±0.11	1.02 ±0.10	12.74 ±9.75	0.96 ±0.09	9.31 ±0.44	4.75** ±0.52	48.97*** ±5.46
4)prevention group n=10	1.38 ±0.11	17.62*** ±1.56	8.23*** ±1.55	53.29*** ±6.12	1.08 ±0.06	1.28 ±0.07	1.01 ±0.04	21.09** ±7.32	0.59 ±0.05	0.72 ±0.05	1.41*** ±0.18	48.93*** ±12.40	1.11 ±0.05	5.02 ±0.61	3.09** ±0.24	38.45*** ±4.58
5)high dose group n=10	1.52 ±0.09	29.75 ±0.96	12.61 ±1.84	57.61 ±4.98	0.98 ±0.10	2.25 ±0.19	1.16*** ±0.06	48.44*** ±9.07	0.65 ±0.06	0.77 ±0.12	1.36*** ±0.22	43.38** ±11.04	1.07 ±0.07	9.42 ±0.50	3.03*** ±0.45	67.83*** ±7.11
	<1>	<2>	<3>		<4>	<5>	<6>		<7>	<8>	<9>		<10>	<11>	<12>	

(Note: HFD = high fat diet; DR = decreasing rate; IR = increasing rate)

*P<0.05   ***P<0.001

<1> Significant difference existed between Group 4 and Groups 1, 2, 3 and 5. <2> Significant difference existed
between Group 4 and the other groups, and between Group 1 and the other groups. <3> Significant difference existed between
Group 4 and Groups 1, 2 and 3, between Group 5 and Groups 1 and 2, and between Group 1 and Groups 2 and 3. <4> Significant
difference existed between Group 4 and the other groups. <5> Significant difference existed between Group 1 and Groups 3, 4

and 5, and between Group 2 and Groups 3, 4 and 5. <6> Significant difference existed between Group 5 and the other groups, and between Group 3 and Groups 1, 2 and 4. <7> Significant difference existed between Group 5 and the other groups, and between Group 3 and Groups 1,2 and 4. <8> Significant difference existed between Group 5 and Groups 1 and 2, and between Group 4 and Groups 1 and 2. <9> Significant difference existed between Group 5 and Group 1. <10> Significant difference existed between Group 4 and the other groups. <11> Significant difference existed between Group 1 and the other groups. and between Group 2 and Groups 4 and 5. <12> Significant difference existed between Group 1 and the other groups, between Group 5 and Group 2 and 4, and between Groups 3 and Group 4.

Table 5. The effect of the extract from pine needle on
the PTH and CT of rats

Groups	N	PTH $\overline{X} \pm$ SD			CT $\overline{X} \pm$ SD		
		before treatment	after treatment	decreasing rate %	after treatment	after treatment	decreasing rate %
(1)normal control group	5	0.34 ±0.03	0.33 ±0.04	2.94 ±0.12	186.5 ±7.69	184.33 ±8.25	-1.03 ±0.05
(2)blank control group	5	0.88 ±0.14	0.95 ±0.12	-7.95 ±0.36	119.50 ±7.56	111.25 ±13.72	-6.90 ±1.22
(3) SP treatment group	5	0.97 ±0.13	0.82 ±0.16	15.46 ±2.51	112.10 ±8.06	136.52 ±13.44	21.78 ±2.96
P value		<0.001	<0.05	<0.05	<0.001	<0.05	<0.001
		Difference existed between Group 1 and Groups 2 and 3.	Difference existed between Group 1 and Groups 2 and 3.	Difference existed between Group 3 and Groups 1 and 2.	Difference existed between Group 1 and Groups 2 and 3.	Difference existed between Group 1 and Groups 2 and 3.	Difference existed between Group 3 and Groups 1 and 2.

Table 6

The test for inhibiting platelet aggregation in vitro					
		1' aggregation rate %(ngEq/ml)		Maximal aggregation rate (Amas)pg/ml	
Groups	n	$\overline{X} \pm$ SD	rate of aggregation-inhibition %	$\overline{X} \pm$ SD	Rate of aggregation-inhibition %
①ADP+phosphate buffer	5	27.16±5.29	.	42.34±2.46	
②ADP+Danshen	5	17.47*±2.9	35.68	26.56*±3.25	38.0
③ADP+Radix Puerariae	5	12.05*±2.0	55.63	22.14*±2.74	48.32
④ADP+pine needle	5	6.91**±1.20	74.56***	15.63*±2.91	63.52**
⑤ADP+compound	5	5.27**±1.13	80.60***	14.13*±2.34	67.02
P value		<0.001		<0.001	
		Difference existed between Group 1 and the other groups. Difference existed between Group 2 and Groups 4 and 5.		Difference existed between Group 1 and the other groups. Difference existed between Group 2 and Groups 4 and 5. Difference existed between Group 3 and Groups 4 and 5.	

*P<0.05
**P<0.01
***P<0.001

Table 7. The effect of the extract from pine needle
on the hemorheology of the patients with hyperviscosity.

| Groups | n | age | ESR | | Haematocrit | | Specific viscosity of plasma | | Specific viscosity of whole blood | | | |
| | | | | | | | | | High shear | | Low shear | |
			before treatment	after treatment	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
Treatment group	87	55.3 ±4.2	28.3 ±3.42	15.23 ±2.61	52.83 ±1.10	45.02** ±1.00	2.02 ±0.10	1.72** ±0.16	6.79 ±0.18	5.88** ±0.17	12.33 ±0.32	7.80** ±0.50
Control group	44	54.6 ±5.6	26.7 ±4.12	20.2* ±3.88	51.16 ±1.22	49.67 ±1.03	1.97 ±0.12	1.84 ±0.06	6.81 ±0.17	6.44** ±0.18	11.89 ±0.57	9.62** ±0.98

*P < 0.05 **P < 0.01 ***P < 0.001

Table 8. The effect of the extract from pine needle on the platelet aggregation rate of the patients with hyperaggregation ($\overline{X} \pm SD$)

Groups	n	1' aggregation rate %			maximal aggregation rate %		
		before treatment	after treatment	inhibition rate %	before treatment	after treatment	Inhibition rate %
treatment group	68	56.33 ±7.33	21.71*** ±3.56	61.46*** ±4.65	87.45 ±11.32	44.37*** ±10.16	49.26*** ±9.64
control group	35	52.65 ±6.46	48.88 ±4.87	7.5 ±1.23	88.06 ±10.25	80.12* ±12.76	9.02* ±3.23

*P<0.05   ***P<0.001

Table 9 The effect of the extract from pine needle on heart rate, TXB_1, PGI_2 of the patients with CHD.

Groups	n	heart rate		TXB_2		PGI_2		TXB_2/PGI_2	
		before treatment	after treatment	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
Treatment group	42	86.44 ±10.62	74.32 ±7.36	163.12 ±81.23	136.27** ±74.36	36.23 ±14.60	157.96*** ±92.65	4.50 ±2.06	0.86*** ±0.47
control group	25	87.6 ±11.24	86.28 ±10.50	156.18 ±83.26	153.06 ±81.54	38.57 ±15.08	55.38* ±15.67	4.05 ±2.13	2.76* ±0.87

*P<0.05   **P<0.01   ***P<0.001

Table 10. The effect of the extract from pine needle on blood-lipid of the patients with CHD.

Groups	n	total cholesterol		triglyceride		high density lipoprotein	
		before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
treatment group	42	6.96±1.06	4.82***±0.92	2.10 ±0.82	1.63*±0.66	1.16±0.30	1.43±0.32
control group	25	6.04±1.13	5.89±1.02	1.98 ±0.76	1.84 ±0.81	1.21±0.36	1.24 ±0.33

*P<0.05   ***P<0.001

Table 11

The effect of the extract from pine needle on ECG of the patients with CHD								
Groups	n	abnormal ECG	remarkable effectiveness		improvement		total effective rate	
			case number	%	case number	%	case number	%
treatment group	42	38	8	21.1	6	42.1	24	63.2
control group	25	21	2	9.5	3	14.3	5	23.8

Table 12

The effect of the extract from pine needle on Serum SOD_1 and Serum lipid peroxide of the senile patients		
	SOD_1(ug/l)	lipid peroxide
before administration	12436.33±9.24.71	9.62±3.40
one month after administration	1162.06±432.64*	7.36±2.58*
three months after administration	411.05±274.38***	5.62±2.13**

*$p<0.05$
**$P<0.01$
***$P<0.001$

Table 13

Comparison of the general condition of patients between the 2 groups							
	sex		age	years old	course of disease (year)		
Groups	male	female		$\overline{X}\pm SD$	<5	5-10	>10
Compositio-n	20	24	43 - 66	53±12.7	24	12	8
Captopril	28	16	39 - 59	47±7.8	24	12	8

Table 14

Comparison the decrements of blood pressure(Kpa) before and after treatment ($\overline{X}\pm SD$)							
	systolic pressure				diastolic pressure		
Groups	case number	before treat-ment	after treat-ment	decrement	before treat-ment	after treat-ment	decrement
composition	41	26.3±3.2	18.9±3.8	7.4±2.9*	16.1±2.1	11.9±3.9	4.2±1.9*.
captopril	44	23.7±5.3	17.1±2.6	6.6±0.7*	14.9±2.9	10.6±2.2	4±2.8*

*$P<0.01$(comparison within the group) (Comparison between the 2 groups) $P<0.05$

Table 15

Comparison of the effects of decreasing the blood pressure before and after treatment between the 2 groups					
Groups	case number	remarkable effective-ness	Effectiveness	ineffectiveness	total effective rate(%)
composition	44	24	10	10	77
captopril	44	26	10	8	82
P>0.05(Comparison between the 2 groups)					

Table 16.

	case number	ECG case number (%)			serum enzyme case number (%)			ESR case number (%)		
		remarkable effectiveness	effectiveness	ineffectiveness	remarkable effectiveness	effectiveness	ineffectiveness	remarkable effectiveness	effectiveness	ineffectiveness
treatment group	16	10*** (63)	5* (41)	1* (6)	12*** (75)	4 (25)	0	9*** (56)	6* (38)	1* (6)
control group	12	5 (41)	4 (33)	3 (26)	8 (67)	3 (25)	1 (8)	5 (41)	5 (41)	2 (18)

*P < 0.05, **P < 0.01, ***P < 0.001

Table 17

The therapeutic effect for the formulation to lower blood pressure.		
	SBP Kpa (mmHg)	DBP Kpa (mmHg)
before treatment	24.4±9.7 (183±72.9)	14.8±0.9 (110.6±6.6)
after treatment	18.7±3.9 (140.3±29.0)	11.6±(86.6±11.9)
Decrement	5.7 (42.8)	3.2 (23.8)
Decreasing rate (%)	23.4	20.5
P	<0.01	<0.01

Table 18

The therapeutic effect of the formulation on hypertension in different periods								
	case number of remarkable effectiveness		case number of effectiveness (%)		total case number of effectiveness(%)		case number of ineffectiveness (%)	
Primary	18	100.0			18	100.0		
Secondary	36	90.0	4	10.0	40	100.0		
Tertiary	2	50.0			2	50.0	2	50.0
Total	56	90.3	4	6.5	60	96.8	2	3.2

Table 19

The therapeutic effect of the formulation on the symptoms and ECG of the patients with angina (n=40)								
	remarkable effectiveness		Effectiveness		total effectiveness		ineffectiveness	
	case number %		case number %		case number %		case number %	
therapeutic effect on symptom	20	50.0	18	15.0	38	95.0	2	5.0
therapeutic effect on ECG	10	25.0	16	40.0	26	65.0	14	35.0

Table 20

The therapeutic effect of the formulation on various angina (n=40)									
The type of angina	case number	remarkable effectiveness		effectiveness		total effectiveness		ineffectiveness	
		case number	%	case number	%	case number	%	case number	%
stable labor pectoris	26	12	46.2	14	53.9	26	100.0		
unstable labor pectoris	14	8	57.1	4	28.6	12	85.7	2	4.3
in total	40	20	50.0	18	45.0	38	95.0	2	5.0

Table 21. The effect of the formulation on blood-lipid and hemorheology (n = 65)

	CH (mmol/L)	TG	HDL-Ch	LDL-Ch %	hema-tocrit	Whole blood specific viscosity (high shear)	plasma specific viscosity (CP)
before treatment	6.4 ±8.2	1.8 ±1.5	1.0 ±0.2	3.6 ±1.3	50 ±0.4	4.95 ±1.15	1.63 ±0.53
after treatment	5.3 ±0.6	1.3 ±0.2	1.5 ±1.1	2.6 ±0.5	45 ±0.5	4.13 ±1.14	1.22 ±0.16
↑ or ↓	1.1↓	0.5↓	0.5↓	1.0↑	5↓	0.82↓	0.41↓
↑ or ↓ (%)	18.0↓	27.2↓	58.8↓	39.2↓	10.0↓	16.6↓	24.6↓
P	<0.05	<0.01	<0.01	<0.01	<0.001	<0.01	<0.01

Claims

1. An extract of pine needle which is in light to dark brown in solide state, water-soluble and has a maximum absorption peak at about 242nm in the ultraviolet-visible light spectrum.

2. An extract of pine needle of Claim 1 for treating diseases including hypertension, CHD, angina pectoris, arrhythemia, myocarditis, diabetes, hyperlipemia, high blood viscosity, high blood aggregation, arterial sclerosis, cerebral infarction, cerebral arteriosclerosis, senile dementia, and sudden deafness.

3. An extract of pine needle of Claim 2 where in the diseases are hypertension, hyperlipemia, high blood viscosity, high blood aggregation, arterial sclerosis, cerebral infarction, cerebral arteriosclerosis, senile dementia, and sudden deafness.

4. An extract of pine needle of Claim 3 where in the diseases are CHD, angina pectoris, arrhythemia, myocarditis, and diabetes.

5. Use of the extract of pine needle of Claim 1 in treating diseases which include hypertension, CHD, angina pectoris, arrhythemia, myocarditis, diabetes, hyperlipemia, high blood viscosity, high blood aggregation, arterial sclerosis, cerebral infarction, cerebral arteriosclerosis, senile dementia and sudden deafness.

6. A method for treating diseases, comprising the administration of a therapeutically effective amount of a pharmaceutical composition containing an extract of pine needle.
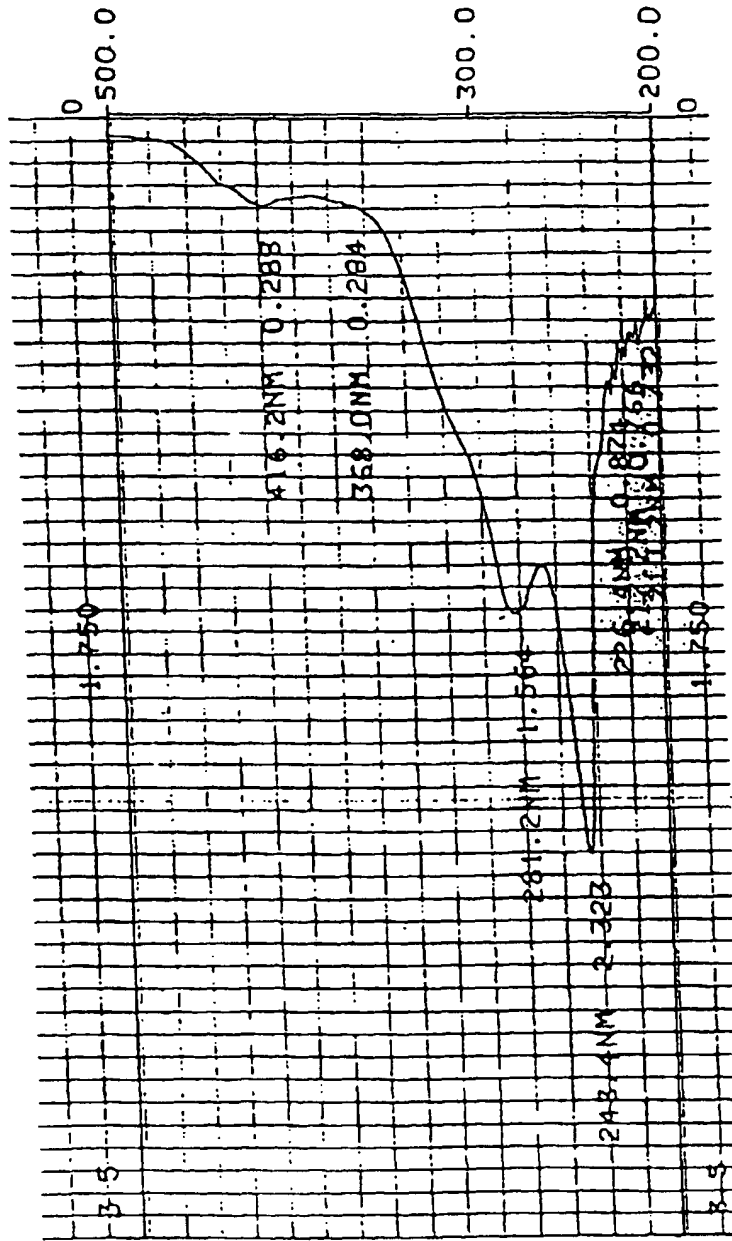
7. A pharmaceutical composition comprising an effective amount of an extract of pine needle and a pharmacologically acceptable carrier or excipient.

8. The pharmaceutical composition of Claim 7, further including an extract of Radix Puerariae wherein the proportion of pine needle and Radix Puerariae is 3:1 — 5:2.

9. The pharmaceutical composition of Claim 7 or 8, further including pearl strutum powder wherein the proportion of pine needle and pearl strutum powder is 20:1 — 2:1.

10. A method for treating diseases including the administration of an therapeutically effective amount of the pharmaceutical composition of Claim 6.

Fig.1

— $R_f = 0.93$

— $R_f = 0.78$

Fig.2

Fig.3a

The expanding effect of the extract from pine needle on

the endothelium-containing aortic circle of SP rats

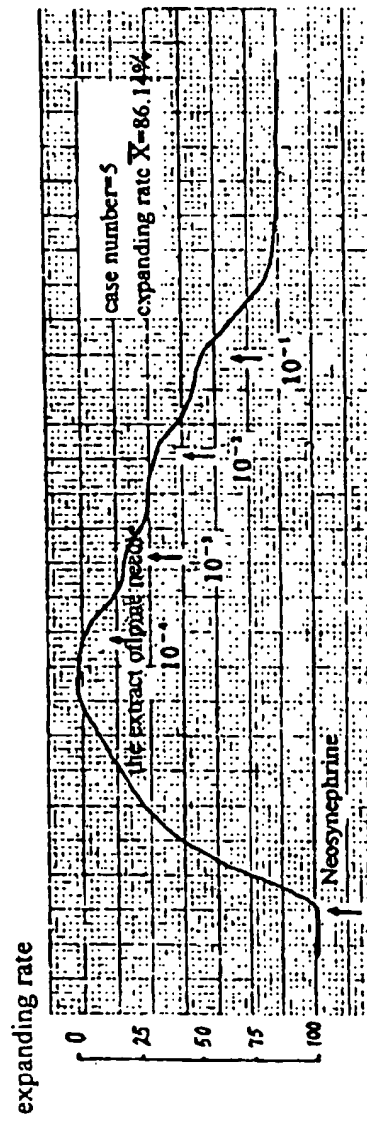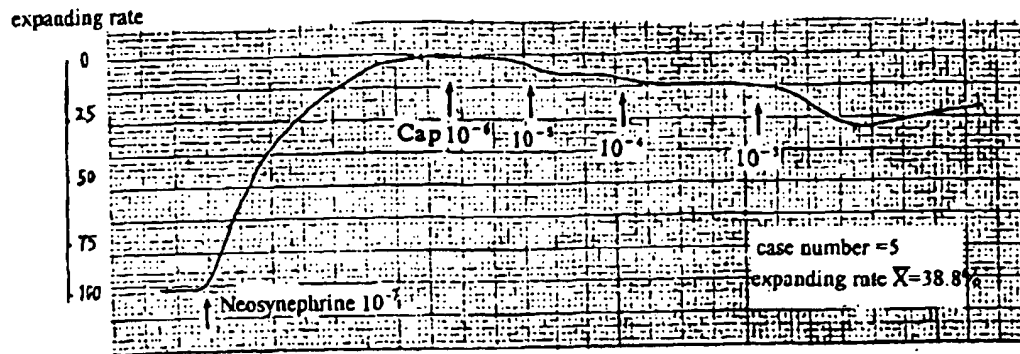Fig.3b The effect of captoril on the endothelium-containing aontic circle of SP rats

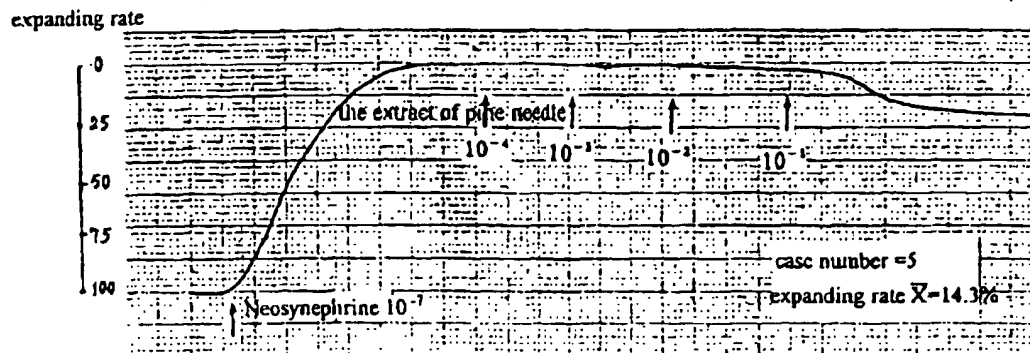Fig.3c The effect of the extract from neeldle on the endothelium free aortic

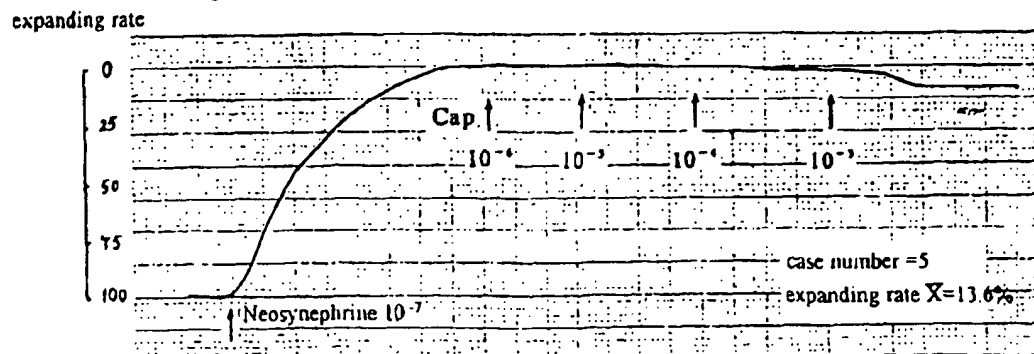Fig.3d The effect of captoril on the endothelium free aortic circle of SP rats
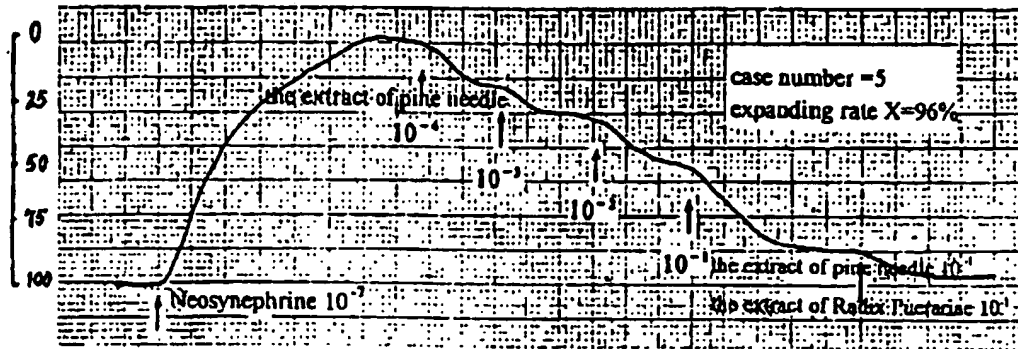
Fig.4   The combined effect for the extract of pine needle
and the extract of Radix Puerariae to expand the blood vessel

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN 97/00006

A. CLASSIFICATION OF SUBJECT MATTER

IPC (6): A61K35/78,35/56

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched(classification system followed by classification symbols)

IPC (6): A61K35/78,35/56

Documentation searched other than minimum documentation to the extent that such documents are included in the field searched

Chinese Patent Documentation,

Electronic data base consulted during the international search(name of data base and, where practicable, search terms used)

WPI(Derwent), CNPAT(CN), JOPAL(JP)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant claim No.
Y	CN, A, 1089142 (Zhang Xiong etc.), 13.JUL.1994(13.07.94)	1 – 10
Y	CN, A, 1099627(Ding Bangyu etc.),08.MAR.1995(08.03.95)	1 – 10
Y	CN, A, 1102111 (Ding Yongnian etc.), 03.MAY.1995(03.05.95)	1 – 10
Y	CN, A, 1097333 (Sun Guofu), 18.JAN.1995(18,01.95)	1 – 10
Y	The effect of pine needle on longevity of vinegar fly, 《 Journal of Zhejiang College of TCM 》,Hu Jun etc.1992,16(6),P49-50.	1 – 10
Y	The study of blood lipid lowering effect of pine needle, 《 Journal of Zhejiang College of TCM 》,Hu Jun etc.1992,16(3),P30-31.	1 – 10

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 · Oct.1997 (15.10.97)	0 6.NOV.1997 (06.11.97)

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